THE PROPERTIES OF THE RECEPTORS IN THE AXON REFLEX SWEATING PRODUCED BY NICOTINE AND SODIUM CHLORIDE*

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In 1939–41, Coon and Rothman (1–3) described that in man intradermal injections of agents with nicotine-like action elicited sweating of the axon reflex mechanism. In their experiments this sweating was inhibited or abolished by procaine and atropine, and in those of Janowitz and Grossman (4) also by tetraethylammonium (TEA).

In a previous paper (5) we observed that a similar sweating was produced by intradermal injection of high concentrations of sodium chloride, and the axon reflex mechanism involved was analyzed precisely by the application of the one band and two band methods and with several axon reflex blocking agents.

The present experiments have extended our previous observations.

EXPERIMENTAL

Methods

The experimental procedures were the same as those described in our previous paper (5). The forearms of healthy males, including the author and his collaborators themselves, as well as medical students of this University, were chosen as test areas. The sweating was visualized by means of the iodine-starch method of Wada and Takagaki (6, 7).

Nineteen blocking agents† were used and their minimal effective concentrations and sites of action for blocking the axon reflex sweating produced by optimal concentrations of nicotine and NaCl were determined using the one band and two band methods. Briefly, a rubber band 3 mm. in width was stretched around the middle part of the forearm to be tested and fastened with a tension sufficient to prevent an axon reflex provoking agent, applied intradermally to either side of the band, from diffusing beyond it to the other side, thereby discriminating between direct stimulation and the axon reflex response. The effect of a given blocking agent on the receptor side of the reflex can be easily determined by application of it by mixing with the axon reflex provoking agent to one side of the band. The effect on the effector side of the reflex can be examined by application of the test agent to the other side of the band. In order to know

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whether the test agent blocks the reflex by acting on the pathway carrying the impulses, it is advantageous to use the two band mtehod. Two rubber bands, about 1 cm. apart and parallel to each other, were tied around the forearm in the same way as described above and the skin between the two bands was infiltrated with the test agent in sufficient width and depth, immediately before application of the axon reflex provoking agent to the area peripheral or central to that demarcated by the bands. The axon reflex sweating was produced by intradermal injections of 0.2 ml. of 10^{-5} nicotine (Merck) and 0.9 % NaCl solutions. Solutions of the test agents to be applied in mixture or separately were made up to contain NaCl at 0.9 per cent, except with the high concentration of NaCl.

Results

The minimal effective concentrations of the blocking agents for completely suppressing the axon reflex sweating, in all of the subjects tested, at the site of the receptors are presented in Table I. As regards sensitivity to the blocking agents, no significant differences were noted between individuals. With these concentrations, the pathway of the axon reflex and the effector points of axons involved remained unaffected. Thus, the blockade of the reflex was proved to have been due to the selective action of the agents on the axon reflex receptors. It should be added that atropine and Banthine in lower concentrations, such as 10^{-7} and 2.5×10^{-7} respectively, paralyzed the receptors of the sweat glands to acetylcholine which might be released from the effector points of axons.

TABLE I

Minimal effective concentrations for causing complete block of axon reflex sweating at the site of receptor of axon

BLOCKING AGENT	AXON REFLEX SWEATING BY	
	10^{-5} nicotine	4% NaCl
KCl	4×10^{-3}	7×10^{-3}
$CaCl_2$	2×10^{-3}	3×10^{-3}
$MgCl_2$	4×10^{-3}	7×10^{-3}
$BaCl_2$	4×10^{-3}	9×10^{-3}
Procaine hydrochloride	2.5×10^{-5}	2.5×10^{-4}
Atropine sulphate	2.5×10^{-5}	2.5×10^{-4}
Banthine bromide	2.5×10^{-6}	2.5×10^{-4}
TEA bromide	1×10^{-3}	(1×10^{-2}: No effect)
l-adrenaline hydrochloride	1×10^{-3}	(1×10^{-3}: Moderate inhibition)
l-ephedrine hydrochloride	1×10^{-4}	5×10^{-3}
Benadryl hydrochloride	1×10^{-4}	1×10^{-4}
Quinine hydrochloride	1×10^{-3}	1×10^{-3}
Quinidine hydrochloride	1×10^{-3}	1×10^{-3}
Chloretone	2×10^{-3}	2×10^{-3}
Pilocarpine hydrochloride	1×10^{-3}	5×10^{-3}
Mecholyl chloride	2×10^{-4}	2×10^{-4}
d-tubocurarine chloride	1×10^{-4}	2×10^{-3}
Hexamethonium bromide	1×10^{-5}	(1×10^{-3}: No effect)
Decamethonium iodide	5×10^{-5}	(1×10^{-3}: No effect)

As the table shows, the axon reflexes produced by both nicotine and NaCl were abolished or inhibited by the blocking agents tested, except TEA, hexamethonium and decamethonium, which blocked the sweat response to nicotine without interfering with the response to NaCl. Although the axon reflex sweating by nicotine and NaCl at the concentrations used was of almost the same extent and duration, the concentrations of procaine, atropine, Banthine, *l*-ephedrine and *d*-tubocurarine required to block the response to NaCl were found to be ten times or more those which abolished the response to nicotine. Similar differences were found with KCl, $CaCl_2$, $MgCl_2$, $BaCl_2$, *l*-adrenaline and pilocarpine. On the other hand, Benadryl, quinine, quinidine, chloretone and Mecholyl at each minimal effective concentration eliminated the sweat responses to both nicotine and NaCl. It seems of interest to note that KCl, $BaCl_2$, *l*-adrenaline, pilocarpine and Mecholyl stimulated directly the sweat glands themselves at the concentrations required to paralyze the axon reflex receptors.

In fact, the properties of the receptors of axon reflex resemble those of the autonomic ganglion cells rather than those of the motor end plates. But, there remain some important differences: potassium ions and pilocarpine which have been said to possess stimulating action on the autonomic ganglion cells, exhibited inhibition, instead of stimulation, of the ability of the receptors to produce axon reflex impulses.

When nicotine dissolved in isotonic glucose (or sucrose) solution at 10^{-5} was injected, the axon reflex sweating failed to occur. This failure was demonstrated to be, under this experimental condition, mainly due to the lack of sodium ions in the external medium of the receptors. Strictly speaking, when the concentration of NaCl in the external medium of the receptors fell below 30 mM/l, the axon reflex was no longer elicited by nicotine in 10^{-5}. It is worth noting that lithium ions could substitute for sodium ions and develop the sweat response to nicotine in the sodium-deficient medium; but, unlike NaCl, LiCl by itself was without any stimulating action on the axon reflex receptors, at a concentration of even 10 per cent. These facts suggest that sodium ions play an important role in the production of the axon reflex impulses.

Furthermore, we have found that the axon reflex sweating can be produced by nicotine and NaCl also in cat's toe pads. This sweating was easily discerned by the one band method. A cotton thread 0.5 mm in diameter was applied transversally around the toe through the middle part of the pad and tightened with an appropriate tension. Intradermal injection of nicotine or NaCl solution on either side of the band resulted in a rapid appearance of the axon reflex sweating on the opposite side of the band. The threshold concentrations of nicotine and NaCl were 10^{-5} and 6 per cent respectively. At about 70 to 76 hours after section of the sciatic nerve or removal of the lumbar sympathetic chain of from the level of the 3rd to that of the 7th lumbar vertebra, the axon reflex could no longer be produced, in spite of the fact that the responsiveness of the cat's sweat glands to direct sudorific actions of adrenaline, Mecholyl and nicotine, was increased by denervation, until its maximum was reached about one week later. This is good evidence that the occurrence of axon reflex sweating depends on the integrity of the sympathetic sweat fibers, and confirms the suggestion of Coon and

Rothman (1–3) that the axon reflex occurs through the ramification of the sweat nerve fibers.

Although the physiological importance of the axon reflex sweating has not been verified, the following possibilities can be considered: the axon reflex sweating may be produced or facilitated by acetylcholine released at the cholinergic endings of the sweat nerve fibers, and by sodium ions, possibly accumulating in the external medium of the axon reflex receptors, during physiological sweating.

SUMMARY

1. The minimal effective concentrations of 19 blocking agents for completely suppressing the axon reflex sweating produced by nicotine and sodium chloride, at the site of the receptors are described.

2. Tetraethylammonium, hexamethonium and decamethonium blocked the sweat response to nicotine without interfering with the response to NaCl.

3. The presence of sodium ions in the external medium of the axon reflex receptors is necessary for eliciting the sweat response to nicotine.

4. The axon reflex sweating was produced also in cat's toe pad by intradermal injection of nicotine and sodium chloride: at about 70 to 76 hours after section of postganglionic sympathetic sweat fibers, the axon reflex sweating could no longer be elicited.

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